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Original Article

Effect of leucine enkephalin administration on ovarian maturation in the freshwater crab *Travancoriana schirnerae*

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Abstract: The current study focused on the effect of administration of the neurotransmitter leucine enkephalin on ovarian maturation in the freshwater crab *Travancoriana schirnerae*. The crabs were administered with leucine enkephalin in multiple doses (10 µl/injection) and their dissected ovaries were processed for histomorphological analyses. Ovarian maturation was assessed by both macroscopic and microscopic observations such as ovarian index, mean oocyte diameter, oocyte proportion values and histological examinations of the ovaries of control and treated crabs. Our observations revealed significantly higher ovarian index, oocyte diameter and oocyte proportion values in treatments over controls. Leucine enkephalin treatment induced previtellogenic ovary to grow into primary vitellogenic, primary vitellogenic to secondary vitellogenic 1 secondary vitellogenic 1 and 2 to secondary vitellogenic 3 stage as evinced by the presence of a large number of primary previtellogenic ovaries in previtellogenic oocytes, larger proportion of secondary vitellogenic oocytes in primary vitellogenic ovaries and secondary vitellogenic stage 3 oocytes in secondary vitellogenic stage 1 ovaries of treated crabs over the controls. The conversion of previtellogenic ovary to early vitellogenic, early to middle and middle to late vitellogenic ovaries in treated crabs probably indicate the stimulatory effect of leucine enkephalin on ovaries either by triggering the release of the gonad stimulating hormone synthesized and released from the brain or thoracic ganglion or by blocking the release of the gonad inhibiting hormone synthesized and secreted by the X organ-sinus gland complex of the eyestalks or both. The results of the present study clearly indicate that leucine enkephalin has a stimulatory effect on ovarian maturation in *T. schirnerae*, thus shortening the period of maturation of ovary, which can be utilized in large-scale production of the species concerned. Further studies are needed to check the efficacy of this neurotransmitter as a supplement in diet to induce ovarian maturation of this species.

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Introduction

Enkephalins are endogenous opioid pentapeptides occurring naturally in the brain of both invertebrates and vertebrates, including crustaceans where they act as neurotransmitters or neuromodulators (Kream et al., 1980; Duve and Thorpe, 1990; Salzet et al., 1997). The presence of enkephalins was reported in retinular cells of the ommatidia (Mancillas et al., 1981; Jaros, 1986), sinus gland, eyestalk ganglia and brain of many crustaceans (Jaros and Keller, 1983). Two structurally different forms of enkephalins were found, namely leucine enkephalin (Leu-Enk) (Simantov and Synder, 1976) and methionine enkephalin (Met-Enk) (Hughes et al., 1975), products of the proenkephalin gene (Gubler et al., 1982; Undenfriend and Kilpatrick, 1983). Leucine enkephalin has the amino acid sequence Tyr-Gly-Gly-Phe-Leu and Met-Enk has the sequence Tyr-Gly-Gly-Phe-Met (Lazarus and Guillemin, 1976). These two pentapeptides bind to morphine receptors in the central nervous system and have opioid properties of relatively short duration (Takahashi, 2016).

Enkephalins enhance the release of neurohormones that control the reproductive activities in both invertebrates (Fingerman, 1987) and vertebrates (Crim et al., 1984). Leucine enkephalin and Met-Enk have antagonistic effects on reproductive indices in crustaceans with Leu-Enk stimulatory in action while

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Met-Enk has an inhibitory role (Kumar et al., 2012). Leucine enkephalin induces the development of gonads either by triggering the production of gonad stimulating hormone (GSH) from the brain or thoracic ganglion or by inhibiting gonad inhibiting hormone (GIH) release from the sinus gland or both (Kulkarni et al., 1981; Reddy, 1991; Sarojini et al., 1995). Methionine enkephalin stimulates the production of GIH or inhibits the production of GSH or both (Fingerman, 1997; Nagaraju, 2011; Swetha et al., 2011). Apart from reproduction, enkephalins are also involved in regulating nociception (Noda et al., 1982; Titus et al., 1989), euphoria, susceptibility to seizures (Bergola et al., 2002), decrease in gastrointestinal motility, cardiovascular regulation and food consumption behaviour through opiate receptors (Micheal et al., 1989; Froehlich, 1997).

Among invertebrates, the role of exogenous enkephalins in reproduction has been studied extensively in decapod crustaceans (Schoofs et al., 1998). Sarojini et al. (1995) observed stimulatory and inhibitory effects of Leu-Enk and Met-Enk in the mud crab, *Uca pugilator*. In the Indian white prawn, *Penaeus indicus*, Reddy et al. (2000) revealed an increase in ovarian index following Leu-Enk administration and a decrease following Met-Enk administration. Influence of Leu-Enk on moulting and vitellogenesis in the freshwater field crab *Oziotelphusa senex senex* was demonstrated by Kishori and Reddy (2003). Stimulation of ovarian growth and vitellogenesis was reported by Kishori et al. (2012) in *O. senex senex* following Leu-Enk administration.

One major problem faced by aquaculture industry is the shortage or non-availability of quality seed as several commercially important species are unable to spawn spontaneously under artificial conditions. Eyestalk ablation (ESA) has been used as the most successful procedure by hatchery industries for induction of moult, gonadal maturation and spawning which often leads to the production of poor quality seed, juveniles and huge mortality of the broodstock. Alternative modes to uphold sustainable aquaculture are the administration of hormones: GSH, methyl farnesoate, ecdysteroids, vertebrate type steroids and stimulatory neurotransmitters either as injections or as feed supplements. Recently, administration of anti GIH antibody which blocks the GIH has been practiced by aquaculture industries for inducing gonadal maturation in captivity. Of these methods, the hormonal manipulation technique is very expensive and thus not cost effective. Moreover, the use of hormones can have potential health hazards to man and the environment. Eating foods contaminated with hormones especially steroid hormones may cause endocrine disorders and development of cancer (Kandarakis et al., 2009). For most crustaceans, the best alternative to hormone injection is the use of neurotransmitters as they have the same effectiveness of hormone injection, cost effective and cause no harmful consequences.

Literature is scanty regarding the role of neurotransmitters on ovarian growth and maturation in freshwater crabs. Further, so far no efforts have been made to study the stimulatory effects of the neurotransmitter Leu-Enk on ovarian growth and maturation in the edible freshwater crab *Travancoriana schirnerae*. In this scenario, the present investigation on the effect of exogenous administration of a stimulatory neurotransmitter on ovarian maturation in this species is attempted. The neurotransmitter, Leu-Enk has been shown to accelerate ovarian maturation by reducing the overall maturation time (Kishori and Reddy, 2003; Kishori et al., 2012), which can be utilized in large-scale production of the species concerned.

Materials and Methods

Adult intermoult females in various phases of oogenesis (previtellogenic, early, middle and late vitellogenic phases) were collected from the paddy fields near Mary Matha Arts and Science College campus, Mananthavady, Wayanad (Kerala, India) during June 2017 to June 2018. They were acclimatized to the conditions of the laboratory for 3 to 4 days. They were fed with pulses, boiled egg and sliced beef liver once in two days. The carapace width, wet weights and moult stages of animals were

recorded.

Leucine enkephalin purchased from Sigma (Sigma Aldrich, St. Louis, MO, USA) was used for injection (1 mg/1 ml distilled water). The crabs were divided into two groups of 10 each. Group I formed the controls and Group II which received leucine enkephalin (10 µl) injections on 1st, 7th, 14th and 21st day through the arthrodial membrane at the base of the coxa of the third walking leg formed the experimentals. Both control and experimental crabs were sacrificed on 30th day. Their ovaries were dissected out; the body weight and wet weight of gonads were recorded to calculate the gonadosomatic index (GSI). One half of the ovary was fixed in Bouin's fluid for histological analysis and the other half used for the measurement of oocyte diameter to determine the stage of development of the ovary. Colour of ovary, oocyte diameter and ovarian index were the criteria used to determine the stage of development of the ovary. Sections were stained with hematoxylin-eosin and observed under a Leica DM 500 Research Microscope. Photomicrographs were taken with a DG 330 /210 camera using Biowizard software.

Mean ovarian index, mean oocyte diameter, oocyte proportion values and histological characteristics of the ovaries of control and treated crabs were the parameters used to evaluate the impact of leucine enkephalin administration on ovarian maturation. The results were calculated as mean±SD and the data obtained for controls and experimentals were subjected to one-way analysis of variance (ANOVA). A value of $P<0.05$ was considered statistically significant.

Results

Morphology of the female reproductive system: The female reproductive system of *T. schirnerae* consists of paired ovaries, oviducts, gonopores and spermathecae. The ovary is H-shaped with anterior and posterior lobes, connected by a transverse bridge of ovarian tissue, located mid-dorsally in the cephalothorax. An oviduct which arises laterally from the posterior part of each ovary extends ventrally and opens out through the coxa of the third walking leg. A pear shaped spermatheca which stores spermatozoa received during copulation was found proximally attached to each oviduct.

Travancoriana schirnerae breeds once in a year, accommodating a single ovarian cycle during the prolonged intermoult phase (Sudha Devi and Smija, 2013). Based on the size and degree of yolk deposition, ten distinct stages were identified in the development of the oocyte: oogonia, chromatin nucleolus (CN) stages 1, 2, 3, perinuclear (PN) stage, primary vitellogenic (PV) stage, secondary vitellogenic (SV) stages 1, 2, 3 and tertiary vitellogenic (TV) stage (Smija and Sudha Devi, 2015). The whole process of oogenesis was divided into six phases: proliferation, previtellogenic, primary vitellogenic, secondary vitellogenic, tertiary vitellogenic and oosorption phases based on morphological and histological features of the ovary.

Morphology and histology of ovary of control and treated crabs during previtellogenic phase (June-September): Morphologically, no remarkable change was noticed in the ovary of treated crabs over controls. They were opaque and cream in colour. On the other hand, injection of leucine enkephalin significantly increased the ovarian index ($P<0.001$) and mean oocyte diameter values ($P<0.001$) of treated females compared to the controls (Table 1). The ovary of experimental crabs showed a predominance of PV oocytes (49%) followed by PN oocytes (30%), CN3 (16%), CN2 (4%) and CN1 (1%) oocytes whereas the control ovary demonstrated larger proportion of PN oocytes (56%) followed by CN3 (29%), CN2 (11%) and CN1 oocytes (4%) (Fig. 1A).

Histological observations of the ovary of control and experimental crabs revealed substantial differences with regard to the pattern of oocyte development. The PN oocytes of injected ovaries were mostly developed into PV oocytes while the control ovaries remained at PN stage. The treated ovaries which reached primary vitellogenic phase contained mainly PV oocytes that occupied towards the periphery of the ovary. The PV oocytes of treated crabs were characterized by the presence of numerous

Table 1. Mean ovarian index and oocyte diameter values of control and treated crabs during different phases of oogenesis.

Phases of oogenesis	Ovarian index		Oocyte diameter	
	Control (µm)	Experimental (µm)	Control (µm)	Experimental (µm)
Previtellogenic phase	0.31±0.04	0.37±0.03**	296.48±0.12	356.77±8.86**
Primary vitellogenic phase	0.53±0.02	0.65±0.10*	436.34±0.41	481.33±4.52**
Secondary vitellogenic phase	2.72±0.38	3.32±0.33*	787.19±9.46	1097.96±15.77**
Tertiary vitellogenic phase	4.48±0.42	4.50±0.38#	1363.60±11.58	1384.60±10.96#

The values are represented as Mean±SD; * P<0.01; ** P<0.001; # not significant

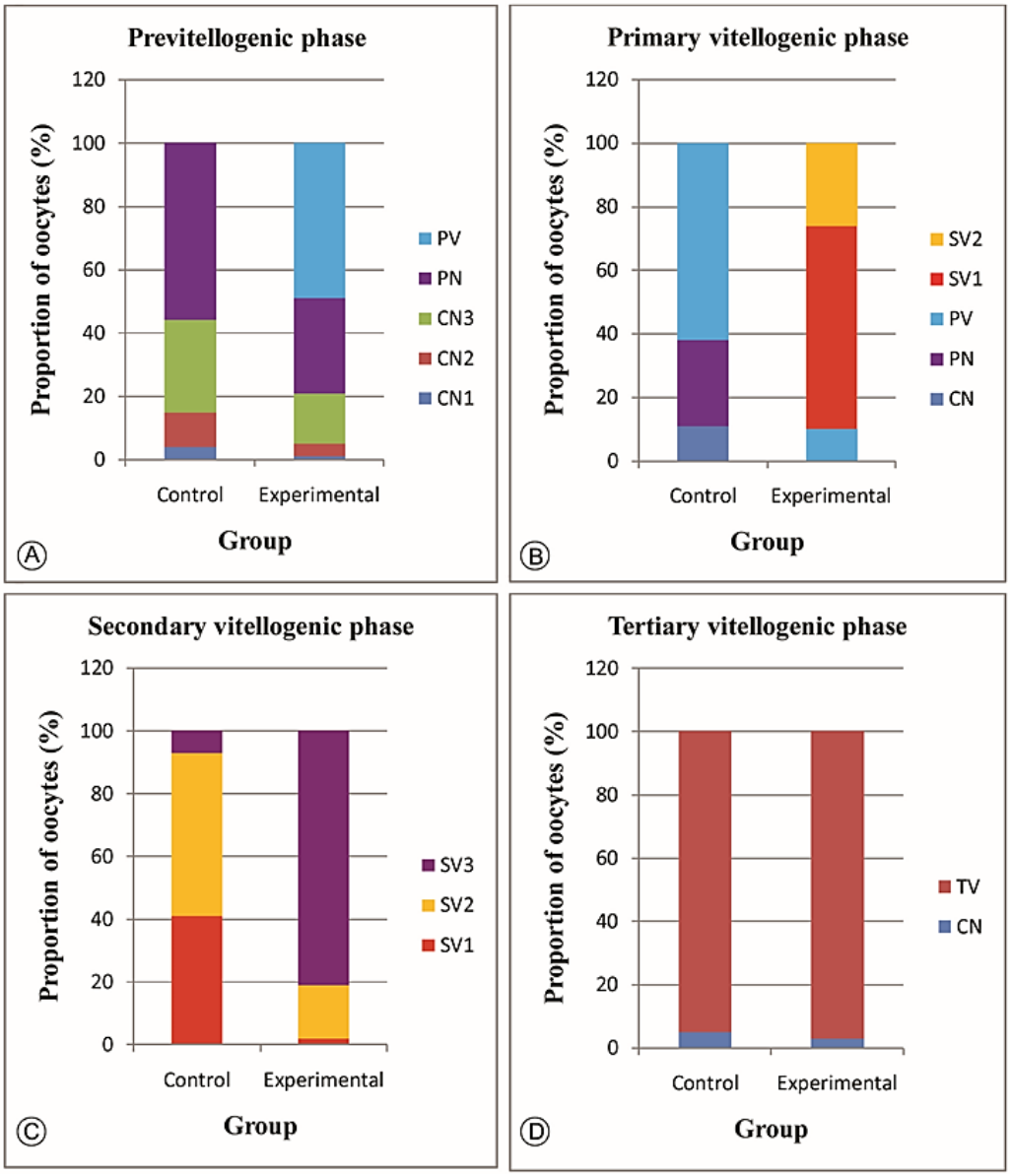

Figure 1. Graph showing the effect of leucine enkephalin on proportion of oocytes during different phases of oogenesis.

large vacuolated globules (19.69±5.16 µm) and yolk globules (15.16±2.18 µm). In addition, some PV oocytes (3%) in treated ovaries showed accumulation of highly basophilic yolk globules in the peripheral ooplasm, which is characteristic of SV1 oocytes. The size of nuclei (55.21±11.10 µm in diameter) and the number (2-4) and size of nucleoli (8.56±2.60 µm in diameter) of PN oocytes were increased considerably in experimental ovaries than the controls (50.32±8.74 µm, 1-2 and 6.14±1.84 µm, respectively). Ovaries injected with leucine enkephalin evinced an increase in the width of perinuclear zone (65.37±16.07 µm) and

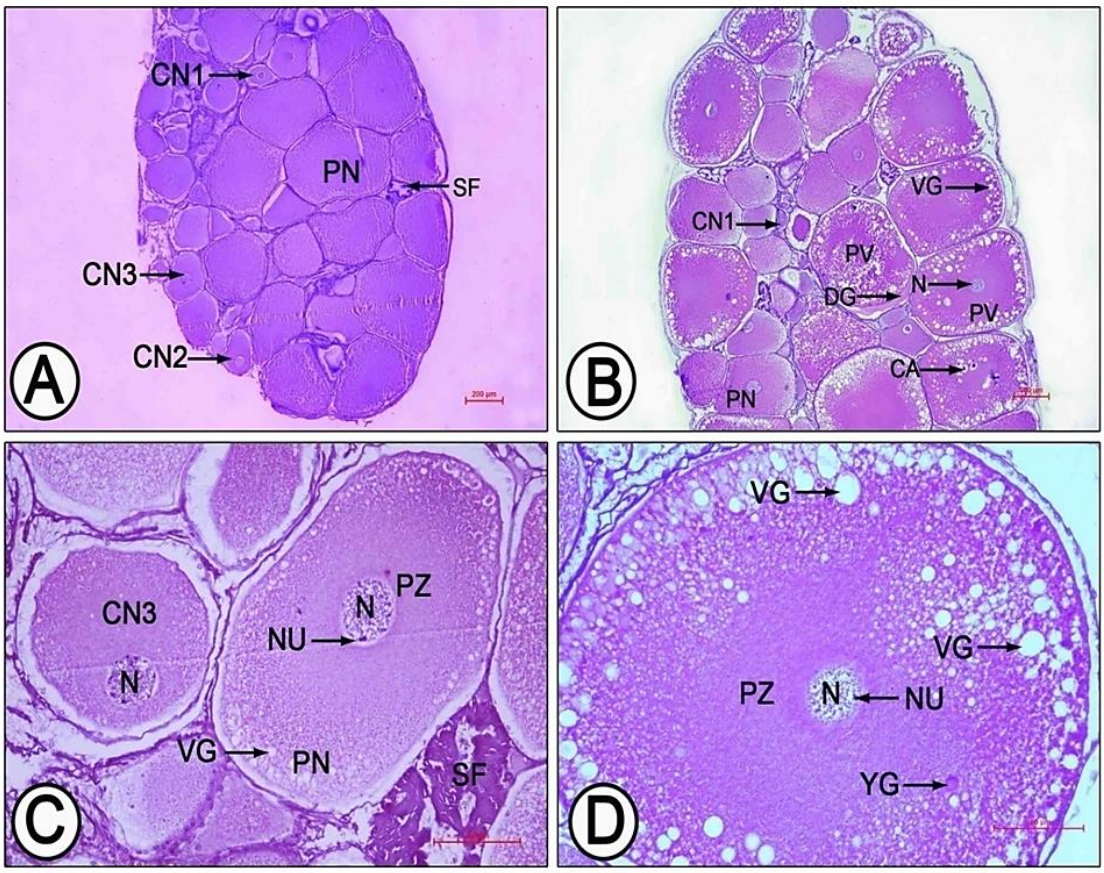

Figure 2. Photomicrograph of control and leucine enkephalin treated ovaries during previtellogenic phase in *Travancoriana schirnerae*. (A) Control ovary dominated by PN and CN oocytes; (B) Treated ovary with a predominance of primary vitellogenic oocytes; (C) Control ovary with PN oocyte at higher magnification; (D) Treated ovary showing PN oocytes with increased perinuclear zone width and large vacuolated globules (CA: Cortical alveoli; CN1: Chromatin nucleolus stage 1 oocyte; CN2: Chromatin nucleolus stage 2 oocyte; CN3: Chromatin nucleolus stage 3 oocyte; DG: Dispersed yolk globule; N: Nucleus; NU: Nucleolus; PN: Perinuclear stage oocyte; PV: Primary vitellogenic stage oocyte; PZ: Perinuclear zone; SF: Shrunken follicle; VG: Vacuolated globule).

number and size of vacuolated globules (13.74±4.26 µm diameter) in the PN oocytes over controls (45.43±18.09 and 11.74±3.26 µm, respectively). Histological examination also revealed that in control crabs, follicle cells were few in number surrounding the CN and PN oocytes whereas treated ovaries indicated the accumulation of more number of follicle cells surrounding the CN and PN oocytes and formation of epithelium around the PV oocytes. The treated ovaries also displayed increased proliferation of oogonia and follicle cells (Fig. 2A-D, Fig. 3A-D).

Morphology and histology of ovary of control and treated crabs during primary vitellogenic phase (October-November): The ovaries of both control and treated crabs were light yellowish in hue. The average ovarian index ($P<0.05$) and the mean oocyte diameter ($P<0.001$) values were significantly high in experimental females compared to the controls (Table 1). The ovaries of treated crabs were dominated by SV oocytes (SV1 64% and SV2 26%) followed by PV oocytes (10%). On the other hand, PV oocytes predominated (62%) the control ovaries with less number of PN (27%) and CN oocytes (11%) (Fig. 1B).

Histological analyses of treated ovaries showed enhanced vitellogenic activities in as much as their ovaries attained secondary vitellogenic phase. The cortical ooplasm of SV1 oocytes was profoundly laden with dense large yolk globules (41.51±9.63 µm in diameter) and vacuolated globules (37.97±8.27 µm diameter). In some SV1 oocytes, the yolk globules were fused to form mildly basophilic yolk platelets, which is characteristic of SV2 oocytes. The SV2 oocytes contained a large store of yolk platelets and vacuolated globules, organized in the entire ooplasm

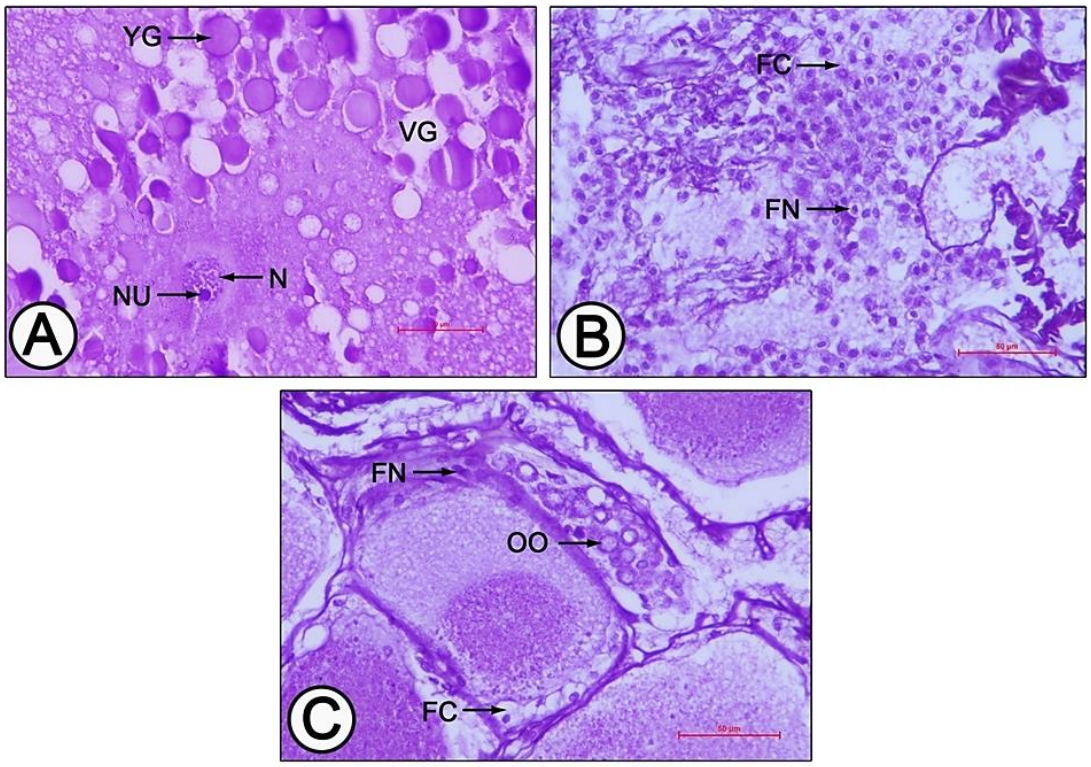

Figure 3. Treated previtellogenic ovary exhibiting follicle and oogonial proliferation and accumulation of yolk globules and vacuolated globules characteristic of SV1 oocytes (A) Primary vitellogenic oocyte displaying accumulation of highly basophilic large yolk globules and vacuolated globules characteristic of SV1 oocytes; (B) Follicle cell proliferation; (C) Oogonial cell proliferation (FC: Follicle cell; FN: Follicle nucleus; N: Nucleus; NU: Nucleolus; OO: Oogonia; VG: Vacuolated globule; YG: Yolk globule).

except the perinuclear zone. In contrast, the ovaries of control crabs showed normal development with large proportion of PV oocytes occupied by small to large yolk globules (14.08±4.28 µm diameter) and vacuolated globules (20.99±4.22 µm diameter) in the peripheral ooplasm. Stimulation of ovarian growth was also evidenced by the proliferation of oogonia and follicle cells in the germinal zone of treated ovaries (Fig. 4 A-D).

Morphology and histology of ovary of control and treated crabs during secondary vitellogenic phase (December-January): Leucine enkephalin treatment did not cause any appreciable change in the ovarian morphology. Both control and treated ovaries were bright yellow in coloration. Administration of leucine enkephalin induced a significant rise in the ovarian index (P<0.05) as compared to the control crabs. Likewise, the treated groups had higher mean oocyte diameter values which were statistically significant (P<0.001) than the controls (Table 1). Leucine enkephalin treatment during secondary vitellogenic phase indicated a greater proportion of SV3 oocytes (81%) with minor proportions of SV2 (17%) and SV1 (2%) oocytes. In contrast, the control ovaries contained mostly SV2 (52%) and SVI (41%) with only a few SV3 (7%) oocytes (Fig. 1C).

The SV3 oocytes contained copious amounts of eosinophilic polygonal yolk platelets (56.14±4.28), narrowing the perinuclear zone to a thin strip. In such oocytes, the nucleus has a shrunken appearance, loses its rounded or spherical form with the advancement of yolk platelets towards the nucleus. There was an increase in the number and size of yolk globules (51.51±7.52 µm diameter) and vacuolated globules (43.97±9.21 µm in diameter) in SV1 oocytes of treated ovaries over controls (41.51±9.63 µm and 37.97±8.27 µm in diameter, respectively). Moreover, the ovaries of injected crabs displayed signs of follicle cell proliferation and growth of younger oocytes (Fig. 5 A-D).

Morphology and histology of ovary of control and treated crabs during tertiary vitellogenic phase

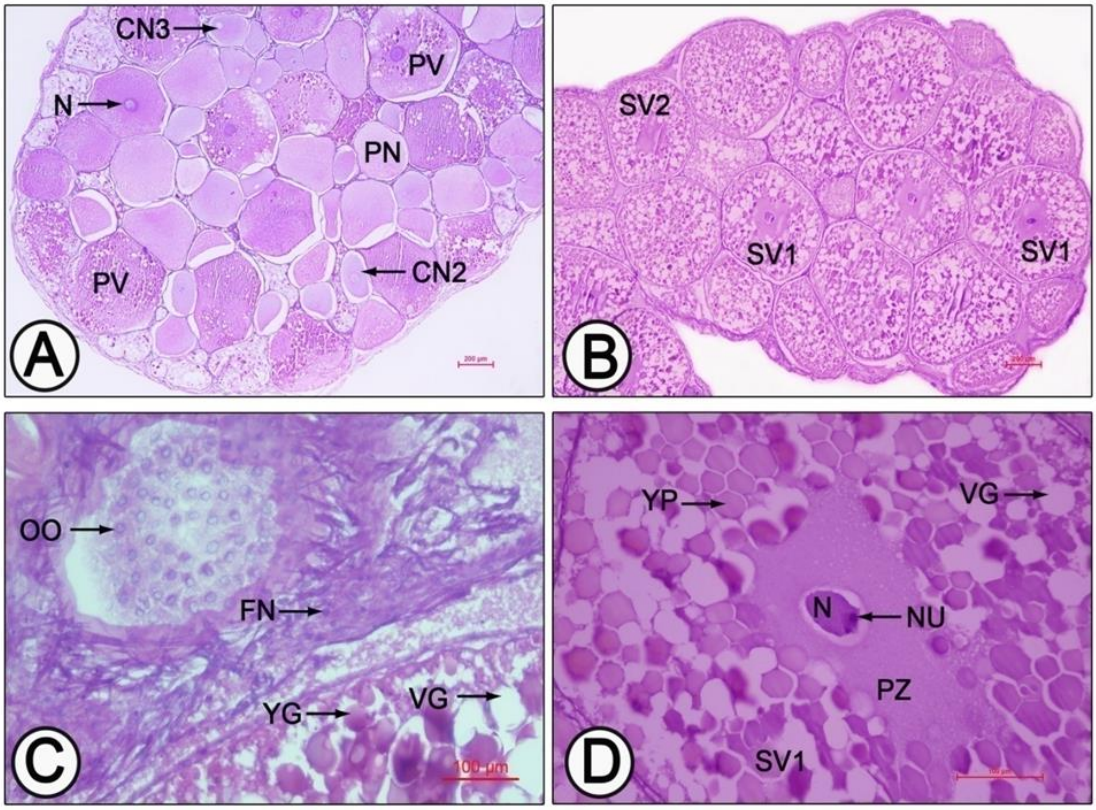

Figure 4. Ovarian histology of control and experimental crabs during primary vitellogenic phase. (A) Control ovary dominated by primary oocytes; (B) Experimental ovary dominated by SV1 and SV2 oocytes; (C) Injected ovary showing oogonial proliferation; (D) Treated SV1 oocytes with yolk platelets, characteristic of SV2 oocytes (CN2: Chromatin nucleolus stage I oocyte; CN3: Chromatin nucleolus stage 3 oocyte; FN: Follicle nucleus; N: Nucleus; NU: Nucleolus; OO: Oogonia; PN: Perinuclear stage oocyte; PV: Primary vitellogenic stage oocyte; PZ: Perinuclear zone; SV1: Secondary vitellogenic stage 1 oocyte; SV2: Secondary vitellogenic stage 2 oocyte; VG: Vacuolated globule; YG: Yolk globule; YP: Yolk platelet).

(February-March): The administration of leucine enkephalin during this phase of vitellogenesis did not result in any significant change neither in the morphology nor in the histology of the ovary. The ovary appeared bright orange in both control and experimental crabs. The mean ovarian index (4.502±0.38) and oocyte diameter values (1384.60±10.96 µm) of the treated crabs were slightly but not significantly, larger than the corresponding values of the control crabs (4.48±0.42 and 1363.44±11.58 µm, respectively) (Table 1).

The ovaries of both control and experimental crabs were compactly filled with a larger proportion of TV oocytes (95 and 97%, respectively) followed by a few CN oocytes (5 and 3%, respectively) (Fig. 1D). The ooplasm of TV oocytes was abundant with eosinophilic yolk platelets which form a homogeneous matrix. The nuclei and nucleoli were not apparent. The follicle cells were no longer visible around the TV oocytes (Fig. 6 A-D).

Discussions

The current study focused on the effect of administration of the neurotransmitter leucine enkephalin on ovarian maturation in the freshwater crab *T. schirnerae*. Ovarian maturation was assessed by both macroscopic and microscopic observations such as ovarian index, mean oocyte diameter, oocyte proportion values and histological examinations of the ovaries of control and treated crabs.

Our observations in *T. schirnerae* revealed no remarkable changes in the ovarian morphology with Leu-Enk treatment when compared to the control ovaries. Similar observations were made by Reddy et al. (2013) in the giant freshwater prawn *Macrobrachium rosenbergii* treated with serotonin. Contrary results were obtained by Kishori and Reddy (2003) and Kishori et al. (2012) in *O. senex senex* and

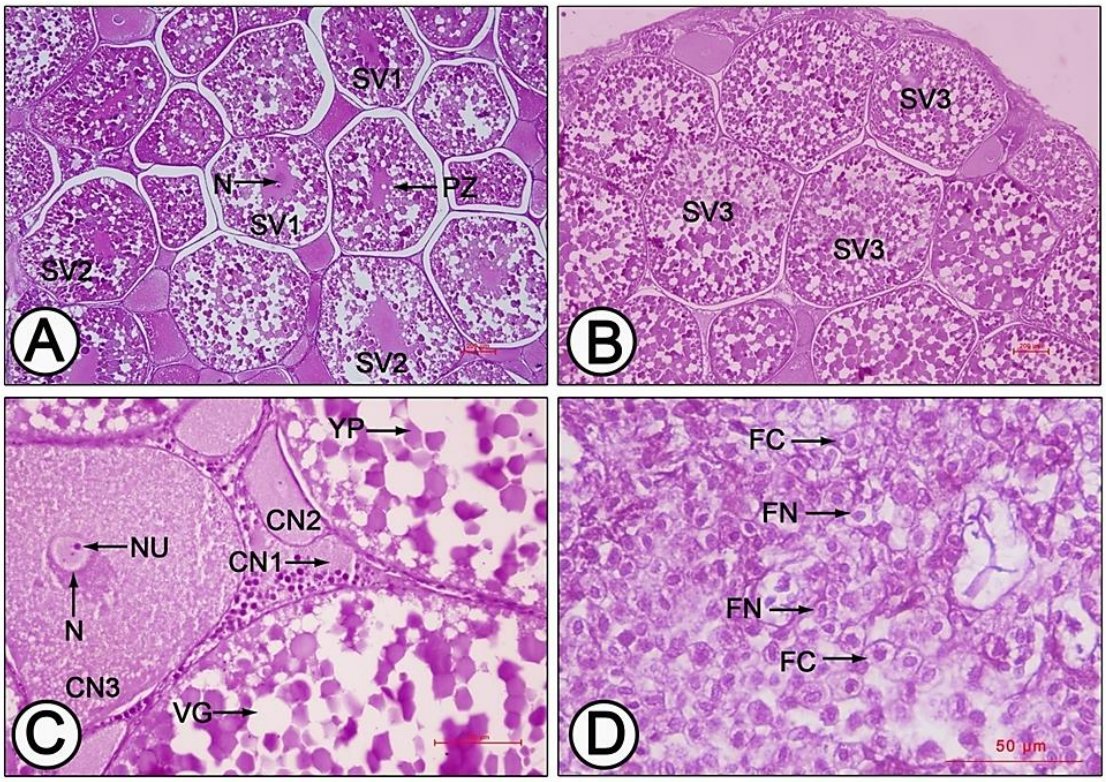

Figure 5. Ovaries of control and experimental crabs during secondary vitellogenic phase. (A) Control ovary with greater proportions of SV1 and SV2 oocytes; (B) Treated ovary dominated by SV3 oocytes; (C) Injected ovary populated by younger oocytes; (D) Treated ovary displaying signs of follicle cell proliferation (CN1: Chromatin nucleolus stage 1 oocyte; CN2: Chromatin nucleolus stage 2 oocyte; CN3: Chromatin nucleolus stage 3 oocyte; FC: Follicle cell; FN: Follicle nucleus; N: Nucleus; NU: Nucleolus; PZ: Perinuclear zone; SV1: Secondary vitellogenic stage 1 oocyte; SV2: Secondary vitellogenic stage 2 oocyte; SV3: Secondary vitellogenic stage 3 oocyte; VG: Vacuolated globule; YP: Yolk platelet).

Reddy (2000) in *P. indicus*.

The present study observed significantly higher ovarian indices in the experimental females than the control crabs. Several authors described increased ovarian index as an outcome of Leu-Enk administration. Kishori and Reddy (2003), Kishori et al. (2012) and Reddy (2000) observed increased ovarian index values in *O. senex senex* and *P. indicus* injected with Leu-Enk. Increased ovarian indices were also reported with other stimulatory neurotransmitters like serotonin, nalaxone, spiperone and octopamine. Treatment of serotonin drastically enhanced the ovarian index value in *U. pugilator* (Richardson et al., 1991), the freshwater crayfish *Procamburus clarkii* (Kulkarni et al., 1992), the freshwater field crab *Paratelphusa hydrodromous* (Ragunathan and Arivazhagan, 1999), white shrimps *Litopenaeus vannamei* and *L. stylirostris* (Vaca and Alfaro, 2000; Alfaro et al., 2004), *P. semisculatus* (Kumulu, 2005), *M. rosenbergii* (Meeratana et al., 2006; Aprajitha et al., 2014), *M. nipponense* (Forsatkar et al., 2013) and the mole crab *Emerita emeritus* (Akhila et al., 2016). Prasad et al. (2014) noticed elevated gonadosomatic index values in the freshwater crab *Barytelphusa guerini*, administered with serotonin and nalaxone. Food containing spiperone and spiperone treatment boosted up the gonadosomatic indices in *P. clarkii* (Rodriguez et al., 2002), *Charybdis granulata* (Zapata et al., 2003), *Aegla platensis* (Cahansky et al., 2008), *A. uruguayana* (Castiglioni et al., 2009) and *Cherax quadricarinatus* (Cahansky et al., 2011). Tinikul et al. (2009) suggested a rise in the ovarian index in *M. rosenbergii* with octopamine treatment. Ovarian index is often used to evaluate ovarian growth and larger ovarian indices are the results of greater number of oocytes developing in response to Leu-Enk injection.

The results of this study clearly showed that Leu-Enk was capable of increasing the mean oocyte diameter values in *T. schirnerae*. Our observations

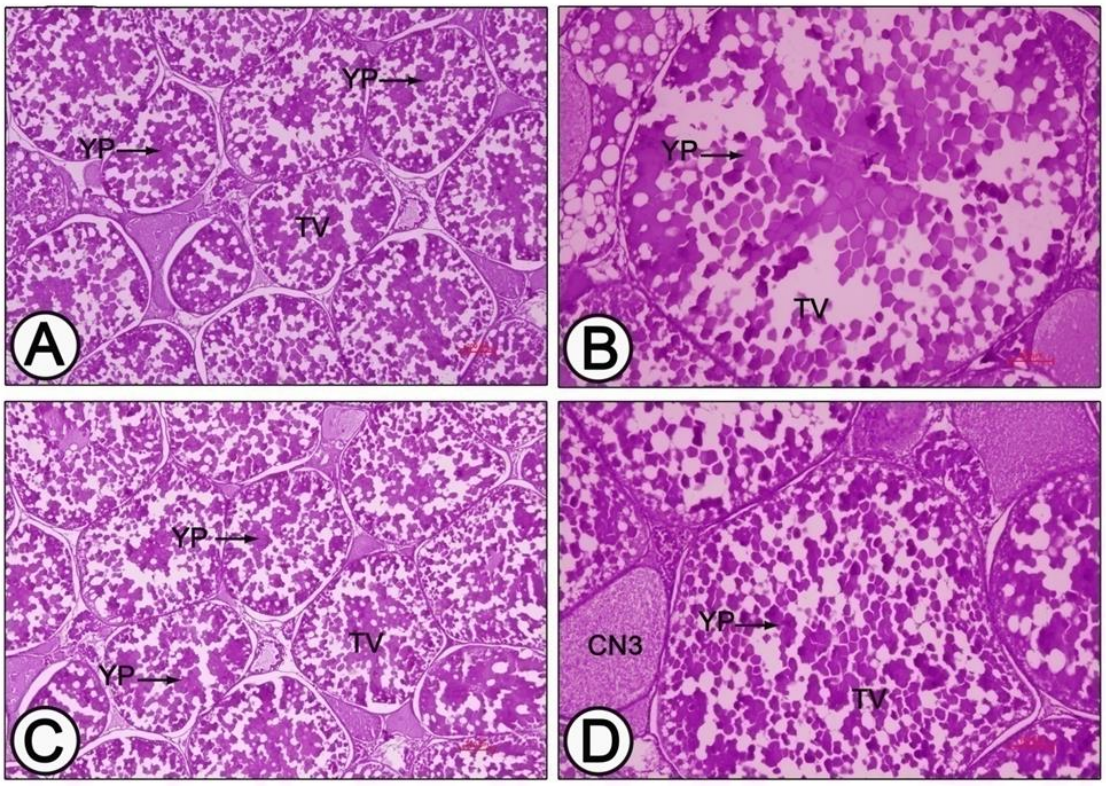

Figure 6. Photomicrograph of ovaries of control and treated crabs during tertiary vitellogenic phase. (A and C) Control and treated ovaries with compactly packed TV oocytes; (B and D) Enlarged view of TV oocytes of control and treated crabs with eosinophilic yolk platelets (CN3: Chromatin nucleolus stage 3 oocyte; TV: Tertiary vitellogenic stage oocyte; YP: Yolk platelet).

were consistent with the findings of Kishori et al. (2012) wherein a significant enhancement in the mean oocyte diameter value was reported for female *O. senex senex* administered with Leu-Enk. Kulkarni et al. (1992), Meeratana et al. (2006) and Akhila et al. (2016) have recorded augmented oocyte diameter values in *P. clarkii*, *M. rosenbergii* and *E. emeritus* treated with serotonin. In *B. guerini*, the diameter of oocytes was significantly increased with serotonin and nalaxone injections (Prasad et al., 2014). Cahansky et al. (2011) observed a significant increase in the oocyte diameter of *C. quadricarinatus* treated with spiperone. By contrast, Kishori and Reddy (2003) demonstrated insignificant mean oocyte diameter values in Leu-Enk treated females of *O. senex senex*. The elevated oocyte diameter value of oocytes in experimental ovaries of the present investigation is suggestive of increased accumulation of lipoproteins and yolk granules in response to Leu-Enk injection.

Leucine enkephalin administration in *T. schirnerae* showed considerable changes in histology of the ovary compared to the controls. Leucine enkephalin treatment induced previtellogenic ovary to grow into primary vitellogenic, primary vitellogenic to SV1 and SV2 and SV1 to SV3 stage as evidenced by the presence of a large number of PV oocytes with yolk globules and vacuolated globules in previtellogenic oocytes, increased width of perinuclear zone in PN oocytes, larger proportion of SV1 and SV2 oocytes in PV ovaries and SV3 oocytes in SV1 ovaries of treated crabs than the controls. This observation was well-supported by the experimental evidence provided by Kishori et al. (2012) in *O. senex senex* that the ovaries of Leu-Enk injected crabs were in vitellogenic stages, confirmed by the accumulation of yolk globules. Reddy et al. (2013) observed that the ovary of serotonin injected *M. rosenbergii* possessed large number of mature oocytes whereas the control crabs exhibited more oogonia and early vitellogenic oocytes. Likewise in *P. hydrodromous*, Ragunathan and Arivazhagan (1999) noticed increased ooplasmic volume with much reduced nucleus in oocytes treated with serotonin. Meeratana et al. (2006) observed a rise in the number of oocytes developing into later stages

of maturation in the ovaries of *M. rosenbergii* injected with serotonin. Babu and Reddy (2014) documented that the ovaries of *P. monodon* treated with serotonin were in the late maturation phase with majority of the oocytes laden with yolk granules. Aprajitha et al. (2014) and Akhila et al. (2016) made observations on the accumulation of large number of yolk globules in ovaries of *M. rosenbergii* and *E. emeritus* treated with serotonin. Chen et al. (2003), Santhoshi et al. (2008) and Reddy et al. (2013) demonstrated increased hemolymph vitellogenin levels in *M. rosenbergii*, *Fenneropenaeus indicus* and *O. senex senex* as a consequence of serotonin injection. The increased accumulation of yolk globules and vacuolated globules, a typical feature of vitellogenesis, in experimental over controls in the current investigation may possibly suggest a positive role for Leu-Enk in the regulation of vitellogenesis.

In *T. schirnerae*, the conversion of previtellogenic ovary to early vitellogenic, early to middle and middle to late vitellogenic ovaries in the treated crabs compared to the controls probably indicate the stimulatory effect of Leu-Enk on the ovaries either by triggering the release of the gonad stimulating hormone (GSH) synthesized and released from the brain or thoracic ganglion or by blocking the release of the gonad inhibiting hormone (GIH) synthesized and secreted by the XO-SG complex of the eyestalks, or both. In decapod crustaceans, ovarian growth and maturation are known to be regulated, directly or indirectly by an array of factors: neurohormones like GSH (Gomez, 1965; Eastman-Reks and Fingerman, 1984) and GIH (Otsu, 1963; Bomirski et al., 1981), terpenoid hormone methyl farnesoate (MF) synthesized and released by the mandibular organ (Laufer et al., 1998), ecdysteroids, synthesized and secreted by the Y organ, vertebrate type steroids (estradiol and progesterone) from the ovary or hepatopancreas (Subramoniam, 2000) and neurotransmitters (both stimulatory and inhibitory). Of these endocrine modulators, the neurotransmitters indirectly influence ovarian growth and maturation by modulating the release of GSH or GIH (Luschen et al., 1993). Stimulatory neuropeptides like Leu-Enk, serotonin, spiperone, nalaxone and octopamine stimulate the release of GSH (Richardson et al., 1991; Kulkarni et al., 1991) and thus stimulate vitellgenesis by acting directly on vitellogenin synthesizing sites (Kulkarni et al., 1981; Sarojini et al., 1995) while inhibitory neurotransmitters like dopamine and Meth-Enk stimulate GIH which in turn act directly on oocytes by blocking vitellogenin uptake and thus yolk protein synthesis (Nagaraju, 2011; Swetha et al., 2011).

Conclusion

The results of the present study clearly indicate that Leu-Enk has a stimulatory effect on ovarian maturation in *T. schirnerae*, thus shortening the period of maturation of ovary. Leucine enkephalin seems to be a cheap and effective alternative to eyestalk ablation or to the use of other endocrine modulators to induce ovarian maturation in species of commercial importance. Further studies are needed to check the efficacy of this neurotransmitter as a supplement in diet to induce ovarian maturation in this species.

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